Molecular Epidemiology Studies on Occupational and Environmental Exposure to Mutagens and Carcinogens, 1997–1999

Molecular epidemiology, bio-environmental exposure, carcinogens

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Molecular epidemiology is a new and evolving area of research, combining laboratory measurement of internal dose, biologically effective dose, biologic effects, and influence of individual susceptibility with epidemiologic methodologies. Biomarkers evaluated were selected according to basic scheme: biomarkers of exposure—metabolites in urine, DNA adducts, protein adducts, and Comet assay parameters; biomarkers of effect—chromosomal aberrations, sister chromatid exchanges, micronuclei, mutations in the hypoxanthine-guanine phosphoribosyltransferase gene, and the activation of oncogenes coding for p53 or p21 proteins as measured on protein levels; biomarkers of susceptibility—genetic polymorphisms of genes *CYP1A1, GSTM1, GSTT1, NAT2*. DNA adducts measured by ^{32}P-postlabeling are the biomarker of choice for the evaluation of exposure to polycyclic aromatic hydrocarbons. Protein adducts are useful as a biomarker for exposure to tobacco smoke (4-aminobiphenyl) or to smaller molecules such as acrylonitrile or 1,3-butadiene. Of the biomarkers of effect, the most common are cytogenetic end points. Epidemiologic studies support the use of chromosomal breakage as a relevant biomarker of cancer risk. The use of the Comet assay and methods analyzing oxidative DNA damage needs reliable validation for human biomonitoring. Until now there have not been sufficient data to interpret the relationship between genotypes, biomarkers of exposure, and biomarkers of effect for assessing the risk of human exposure to mutagens and carcinogens. *Key words*: air pollution, benzene, biomarkers, coke oven, genotypes, occupational exposure, PAHs, pesticides, tobacco smoke. — *Environ Health Perspect* 108(suppl 1):57–70 (2000). *http://ehpnet1.niehs.nih.gov/docs/2000/suppl-1/57-70sram/abstract.html*

A new and evolving area of research termed molecular epidemiology attempts to merge sophisticated and highly sensitive laboratory methods (many of them developed during the recent revolution in molecular biology) with analytical epidemiologic methods. Molecular epidemiology bridges basic research in molecular biology and studies of human cancer causation by combining laboratory measurement of internal dose, biologically effective dose, biologic effects, and the influence of individual susceptibility with epidemiologic methodologies (*1*). The most common view is that the approach represents a natural convergence of molecular biology and epidemiology (*2*).

The number of biomarkers available for evaluating genetic and cancer risk in humans is quite large. Their utility for human biomonitoring is suggested by the well-known paradigm of environmentally induced cancer, which represents end points for assessing the entire spectrum of human–genotoxicant interactions (*3*). These biomarkers begin with exposure and include absorption, metabolism, distribution, critical target interaction (i.e., DNA damage and repair), genetic changes, and finally, disease, which is the province of traditional epidemiology. The development of biomarkers has given rise to the field of molecular epidemiology, which uses these biomarkers rather than disease to assess the risk of environmental exposure (*4,5*).

The paradigm of environmental cancer starts with exposure. A large number of biomarkers are now available, but to evaluate their sensitivity and to interpret the results obtained, data on exposure are needed. Previously, exposure data were usually not published in studies using various biomarkers.

The field of molecular epidemiology is developing rapidly. This review covers papers published from 1997 to 1999. The data can be used to evaluate the advantages or disadvantages of different biomarkers used in the studies of occupationally and environmentally exposed population groups.

Biomarkers evaluated in this review were selected according to the following basic scheme: biomarkers of exposure—metabolites in urine, DNA adducts, protein adducts, and Comet assay parameters; biomarkers of effect—chromosomal aberrations (CAs), sister chromatid exchanges (SCEs), micronuclei (MN), mutations in the hypoxanthine–guanine phosphoribosyltransferase (HPRT) gene, and the activation of oncogenes coding for p53 or p21 proteins as measured by protein levels.

Occupational Exposure to Mutagens and Carcinogens

Table 1 summarizes the effect of exposure and genotypes on biomarkers of exposure and the effects in occupationally exposed groups.

Several studies on coke oven workers have used personal monitoring. In a group of coke oven workers exposed to carcinogenic polycyclic aromatic hydrocarbons (PAHs) from 0.6 to 547 μg/m^3 and to benzo[*a*]pyrene (B[*a*]P) from 2 to 62,107 ng/m^3, respectively, Binková et al. (*6*) observed a positive correlation between DNA adducts in total white blood cells (WBCs) and/or lymphocytes and carcinogenic PAHs and/or B[*a*]P in the inhaled air at the individual level. A similar relationship in the same groups was also observed by Kalina et al. (*7*) who analyzed CAs, SCEs, and cells carrying a high frequency of SCEs (HFCs). Using the Comet assay for lymphocytes, Mračková et al. found no effect (*8*). In a study of coke oven workers in China (*9*) and Taiwan (*10*), urinary 1-hydroxypyrene (1-OH-pyrene) differed significantly according to exposure to PAHs, but no increase of DNA adduct levels between exposed and control groups was observed (*9*). Personal exposures to B[*a*]P in this study were 2 or 3 times higher than in the former study (*6*). Kure et al. (*11*) analyzed benzo[*a*]pyrene diolepoxide (BPDE)-albumin adducts in coke oven workers. They found no difference between exposed and control groups. The concentration of B[*a*]P in the workers' environment was approximately only one-third to one-half the exposure in the first study (*6*). Zanesi et al. (*12*) measured HPRT mutant frequency and analyzed mutational spectra in the lymphocyte mutants. The gene alterations observed were similar in exposed and nonexposed subjects.

Pendzich et al. (*13*) also studied the effect of exposure on SCEs and HFCs in coke oven workers. SCEs, as well as HFCs, were significantly higher in coke oven workers than in controls from the same region. The winter samples had higher SCEs and HFCs than the summer samples. Differences between smokers and nonsmokers were observed, particularly in winter samples.

Analysis of aromatic DNA adducts in foundry workers and controls showed that the ^{32}P-postlabeling method was able to

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This research was supported by a grant from the Czech Ministry of Environment, Teplice Program II.

Received 29 September 1999; accepted 3 December 1999.

PM3006440593

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Table 1. Effect of exposure and genotypes on biomarkers of exposure and effects by occupationally exposed groups.

Group/ sample size	Exposure		Effect of exposure on biomarkers									Effect of biomarkers of susceptibility	Ref.
	Type	Measured	Urine[a]	DNA adducts[b]	Protein adducts[c]	Comet	CAs	SCEs	MN	HPRT	p53/p21 proteins		
Coke oven workes													
Exp = 68; C = 56	PAHs	Personal	–	E	–	–	–	–	–	–	–	*GSTM1* No E *NAT2* No E	(6)
Exp = 65; C = 34	PAHs	Personal	–	–	–	–	E	E	–	–	–	*GSTM1* No E *NAT2* No E	(7)
Exp = 24; C = 28	PAHs	Personal	–	–	–	No E	–	–	–	–	–	*GSTM1* No E *NAT2* No E	(8)
Exp = 75; C = 24	PAHs	Personal, ambient	E	No E	–	–	–	–	–	–	E	*CYP1A1* E *GSTM1* No E	(9)
Exp = 80	PAHs	Personal	E	–	–	–	–	–	–	–	–	*CYP1A1* E	(10)
Exp = 59; C = 48	PAHs	Personal	–	–	No E	–	–	–	–	–	–	–	(11)
Exp = 43; C = 26	PAHs	No	–	–	–	–	–	–	–	No E	–	–	(12)
Exp = 45; C = 38	PAHs	No	–	–	–	–	–	E	–	–	–	–	(13)
Foundry workers	PAHs												
Exp = 95		Personal	–	E	–	–	–	–	–	–	–	*CYP1A1* No E *GSTM1* No E	(14)
Engine workshop	Engine exhaust, PAHs												
Exp = 61; C = 30		No	E	–	–	–	–	E	E	–	–	–	(18)
Workers	Bitumen fumes, PAHs												
Exp = 28; C = 28		No	E	–	–	–	–	E	E	–	–	–	(19)
Soldiers	Oil well fires, PAHs												
Exp = 20; C = 33		Personal	No E	No E	–	–	–	–	–	–	–	*CYP1A1* No E *GSTM1* No E *GSTT1* No E	(20)
Bus garage workers	Diesel exhaust, nitro-PAHs												
Exp = 29; C = 26		No	–	–	No E	–	–	–	–	–	–	–	(21)
Airport personnel	VOCs												
Exp = 39; C = 11		Personal	–	–	–	No E	–	No E	No E	–	No E	–	(23)
Workers	Traffic fumes, VOCs												
Exp = 32; C = 29		Personal	–	–	–	–	–	–	No E	–	–	–	(24)
Workers	Hydrocarbons												
Exp = 44; C = 24		No	–	–	–	E	E	No E	–	–	–	–	(25)
Gasoline station attendants													
Exp = 12; C = 12	Benzene	Personal	–	–	–	–	–	–	No E	–	–	–	(26)
Exp = 12; C = 12	Benzene	Personal	–	–	–	E	–	–	–	–	–	–	(27)
Exp = 22; C = 19	Benzene	Personal	–	–	–	–	–	No E	E	–	–	–	(28)
Exp = 45; C = 45	Benzene	Personal	–	–	–	–	–	No E	–	–	–	–	(22)
Workers													
Exp = 43; C = 42	Benzene	Personal	–	–	E	–	–	–	–	–	–	–	(30)
Exp = 43; C = 44	Benzene	Personal	–	–	–	–	E	–	–	–	–	–	(31)
Exp = 43; C = 44	Benzene	Personal	–	–	–	–	E	–	–	–	–	–	(32)
Exp = 35; C = 13	Benzene	Personal	–	–	–	–	–	–	No E	–	–	–	(33)
Exp = 34; C = 19	Organic solvents	Ambient	–	–	–	No E	–	–	–	–	–	*GSTM1* No E *GSTT1* No E	(34)
Viscose rayon plant workers	Acrylonitrile + dimethyl formamide												
Exp = 26; C = 26		No	–	–	–	–	E	E-HFC	–	–	–	–	(35)
Furniture workers	Styrene												
Exp = 53; C = 41		Ambient	E	–	–	–	–	E	No E	–	–	–	(36)

(*Continued*)

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Table 1. (*Continued*)

Group/ sample size	Exposure Type	Exposure Measured	Effect of exposure on biomarkers: Urine[a]	DNA adducts[b]	Protein adducts[c]	Comet	CAs	SCEs	MN	HPRT	p53/p21 proteins	Effect of biomarkers of susceptibility	Ref.
Workers													
Exp = 19; C = 19	1,3-Butadiene	Personal	–	–	–	No E	E	E	No E	–	–	–	(37)
Exp = 15; C = 11	1,3-Butadiene	Personal	–	E	–	–	–	–	–	–	–	–	(38)
Exp = 24; C = 19	1,3-Butadiene	Personal	–	–	–	–	E[d]	–	–	–	–	–	(39)
Exp = 14; C = 14	Epichlorohydrin	Personal	–	–	No E	–	–	E	No E	No E	–	–	(40)
Farmers													
Exp = 20; C = 20	Pesticides	No	–	–	–	–	E[d]	–	–	–	–	*CYP2E1* E[d] *GSTM1* E[d] *GSTT1* No E *PON* E[d]	(41)
Exp = 29; C = 11	Pesticides	No	–	–	–	E	–	–	–	–	–	–	(42)
Exp = 40	Pesticides	No	–	–	–	E	–	–	–	–	–	–	(43)
Greenhouse workers													
Exp = 34; C = 33	Pesticides	No	–	–	–	–	–	–	E	–	–	*GSTM1* No E *NAT2* No E	(44)
Exp = 57; C = 33	Pesticides	No	–	E	–	–	–	–	–	–		–	(45)
Exp = 30; C = 32	Pesticides	No	–	–	–	–	No E	–	–		–	*GSTM1* No E *GSTT1* No E	(56)
Sprayers	Malathion												
Exp = 38; C = 16		No	–	–	–	–	–	–	No E	–	–	–	(46)
Phosphate–fertilizers	HF, SiF												
Exp = 40; C = 40		No	–	–	–	–	E	–	E	–	–	–	(47)
Welders	Cr, Ni												
Exp = 39; C = 39		No	–	–	–	–	–	E	–	–	–	–	(48)
Nurses													
Exp = 20; C = 11	Cytostatics	No	–	–	–	–	E	–	–	–	–	–	(49)
Exp = 26; C = 14	Cytostatics	No	–	–	–	–	–	–	E	–	–	–	(50)
Exp = 30; C = 30	Cytostatics	No	–	–	–	E	–	–	–	–	–	–	(51)
Operating room personnel	Anesthetic gases												
Exp = 66; C = 41		No	–	–	–	E	–	–	–	–	–	–	(52)
Workers	Tobacco dust												
Exp = 107; C = 41		No	–	–	–	E	–	–	–	–	–	–	(53)
Pilots + crews													
Exp = 192; C = 55	Cosmic radiation	No	–	–	–	–	E	–	E	–	–	–	(54)
Exp = 23; C = 23	Cosmic radiation	No	–	E	–	–	No E	–	No E	No E	–	–	(55)

Abbreviations: –, biomarker was not analyzed; C, number of subjects in control group; Comet, Comet assay; E, statistically significant effect of exposure and/or genotypes on biomarkers of exposure and effect; Exp, number of subjects in exposed group; HPRT, mutations in the hypoxanthine–guanine phosphoribosyltransferase gene; p53/p21, activation of oncogenes coding for p53 or p21 proteins. [a]Metabolites in urine. [b]By ^{32}P-postlabeling or ELISA. [c]Hemoglobin or albumin adducts. [d]Effect on CAs only using challenge assay.

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detect an increase of DNA adducts in leukocytes when exposure to B[*a*]P in the air exceeded 5 ng/m^3 (*14*).

Coke oven and foundry workers certainly represent a group of subjects highly exposed to PAHs (*6–13*). Using biomarkers of exposure such as DNA adducts (*6,13*) and 1-OH-pyrene (*9*), significantly higher levels of PAHs were seen in these workers compared with levels in control groups. However, Binková et al. (*6*) evaluated the efficiency of DNA adduct formation per unit of exposure and found that the efficiency decreased remarkably with increasing exposure. An increase of exposure to PAHs of approximately 40-fold resulted in only a 50% increase of bulky aromatic DNA adducts in lymphocytes. Similar results showing a nonlinear dose response were described by Lewtas et al. (*15*) for environmentally and occupationally exposed subjects and by Van Schooten et al. (*16*) for smokers. This observation needs further investigation, but it could be caused by the saturation of metabolic processes, a lack of specificity of the assay in the control population, or a lack of transport of the activated genotoxins to the tissue under study (*17*).

Young workers in engine repair workshops are exposed to PAHs from engine exhaust and used engine oil. SCE and MN frequencies were found to be higher for exposed subjects ($p < 0.05$). The levels of 1-OH-pyrene were higher not only in the exposed group compared with controls ($p < 0.001$) but also in exposed nonsmokers compared with exposed smokers ($p < 0.05$) (*18*).

Workers employed in road-paving operations are exposed to bitumen fumes, which consist mainly of PAHs and their derivatives. Exposure to PAHs has been assessed by 1-OH-pyrene excretion in the urine: 0.78 ± 0.46 µmol/mol creatinine in exposed workers versus 0.52 ± 0.44 µmol/mol in controls. Exposure to bitumen fumes significantly increased SCEs ($p < 0.05$) and MN ($p < 0.001$) in exposed workers compared with controls (*19*).

A possible effect of PAHs was studied in U.S. soldiers in Kuwait who were exposed to oil-well fires. The PAH–DNA adducts were measured in blood cells by immunoassay. Low ambient PAH levels were observed, and no increases of DNA adducts or 1-OH-pyrene in urine were found (*20*).

Gas chromatography–mass spectrometry (GC–MS) was used to analyze hemoglobin adducts formed by nitro-PAHs in human blood samples of a population of bus garage workers exposed to diesel exhaust. When blood samples from bus garage workers were compared with those from urban area controls, no significant differences in hemoglobin adduct levels were observed (*21*).

The genotoxicity of a low level of hydrocarbons and jet fuel derivatives was studied in airport workers. The levels of benzene, toluene, and xylene at the airport were approximately one-tenth the levels of those at petrol stations (*22*). Analyzing SCEs, MN, Comet assay parameters, and the induction of *ras* p21 protein levels in plasma, no effect of this low exposure to hydrocarbons was observed. Smoking did not significantly affect the Comet assay values. The study suggests that benzene at very low doses (0.10 ± 0.005 mg/m^3) does not induce detectable genetic damage as measured by conventional cytogenetic assays (*23*).

Parry et al. (*24*) analyzed the effect of vehicle exhaust fumes on motor mechanics, traffic policemen, and motorcyclists. Controls were office workers. Using diffusion tubes, the exposure to volatile organic compounds (VOCs) was determined. No increase of micronuclei was observed when the total VOC was, on average, 363 ppb in the exposed group versus 138 ppb in controls (exposure to benzene 0.041 mg/m^3 for the exposed group; 0.016 mg/m^3 in controls).

Hartmann et al. (*25*) analyzed workers exposed to environmental pollutants at a waste disposal site. Using a cytogenetic test and the Comet assay, they observed an increase of CAs and Comet assay parameters but not of SCEs. Expected exposure was to various hydrocarbons (e.g., acrylonitrile, benzene, dinitrotoluene, epichlorohydrine, vinylchloride).

Gasoline station attendants represent a group of persons exposed to benzene. Carere et al. (*26*), using fluorescence *in situ* hybridization (FISH) with centromeric probes for chromosomes 7, 11, 18, and X, observed no exposure-related hyperploidy or micronucleus formation for a group exposed to benzene concentrations of 0.32 mg/m^3. Applying the Comet assay to the same groups, Andreoli et al. (*27*) found that tail moment values were significantly higher in exposed groups than in unexposed groups (*27*). Bukvic et al. (*28*) found no effect on SCEs or HFCs of exposure to benzene at concentrations of 0.23 mg/m^3. They only observed an exposure-related increase in the frequency of micronuclei. In another group of service station attendants exposed to 0.19 mg/m^3 benzene, no differences in SCE values were observed among exposed and control groups (*22*).

Exposure to benzene has been followed in several groups, as benzene may be used as a model for biomarkers of leukemia risk (*29*). In Shanghai, China, two groups of workers were studied, one with a lower exposure (50 ± 31 mg/m^3) and one with a higher exposure (380 ± 253 mg/m^3) to benzene versus controls exposed to 0.05 ± 0.06 mg/m^3. Hemoglobin and albumin adducts significantly correlated with exposure (*30*). Both these adducts therefore may be used as biomarkers of exposure to high levels of benzene. Smith et al. (*31*) used painting probes for chromosomes 8 and 21. They observed an increase in the hyperdiploidy of chromosomes 8 and 21 and translocations between chromosomes 8 and 21 with exposure to benzene concentrations higher than 380 mg/m^3. Zhang et al. (*32*) used FISH in the same group to determine specific aberrations in chromosomes 1, 5 and 7. Exposure to benzene was associated with increases in the rates of monosomy 5 and 7 and with increases in the trisomy and tetrasomy frequencies of all three chromosomes. This result demonstrates that the leukemia-specific changes in chromosomes 5, and 7 can be detected by FISH in the peripheral blood of healthy exposed workers.

Surrallés et al. (*33*) used FISH to determine MN in lymphocytes and buccal cells as well as numerical abnormalities of chromosome 9 in buccal cells in a population occupationally exposed to approximately 3.5 mg/m^3 benzene in an Estonian petrochemical plant. No increases in the frequency of total micronuclei or chromosome 9 numerical abnormalities were detected in either buccal cells or lymphocytes.

Pitarque et al. (*34*) used the Comet assay to evaluate the exposure to organic solvents such as acetone, gasoline and toluene in shoe workers. The occupational exposure to organic solvents (acetone, 382–927 mg/m^3; gasoline, 283–723 mg/m^3; toluene, 96–412 mg/m^3) did not affect the Comet assay values (*34*).

Major et al. (*35*) followed a group of viscose rayon plant workers exposed to acrylonitrile (0.3–17.6 mg/m^3 in ambient air) and dimethylformamide (0.6–23 mg/m^3). In exposed workers, increased levels of CAs, SCEs, and HFCs, as well as ultraviolet-induced unscheduled DNA synthesis, were observed (*35*). In furniture workers exposed to styrene, exposure at an ambient level of 128 mg/m^3 of styrene increased SCE frequency but not MN (*36*).

Exposure to 1,3-butadiene (BD) at a level of 0.53 mg/m^3 in a monomer production unit increased CAs, SCEs, and HFCs in the exposed group compared with levels among controls but had no significant effect on the formation of MN or Comet assay parameters (*37*). Zhao et al. (*38*), in a subgroup of the same workers, were able to demonstrate an increase of *N*-1-(2,4,3-trihydroxybutyl)adenine adducts induced by BD. Hallberg et al. (*39*) observed no differences in the frequency of CAs between the exposed group with occupational exposure to 5 mg/m^3 BD and controls. Using a challenge assay, they postulated that BD could cause DNA repair defects.

Exposure to epichlorohydrin (ECH) (0.4–0.9 mg/m^3) increased SCEs and HFCs

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in the exposed group compared with controls. These concentrations of ECH did not increase hemoglobin adducts, HPRT mutants, or MN (*40*).

Another group studied was farmers exposed to a mixture of pesticides. Sometimes it is difficult to identify individual pesticides and to properly evaluate the exposure to them. Au et al. (*41*), analyzing the difference between farmers and controls, did not observe any increase in CAs by either the standard assay or FISH; differences were apparent, however, using a challenge assay. Lebailly et al. (*42*) observed effects of spraying selected pesticides on several groups using the Comet assay. DNA damage in leukocytes was analyzed during the beginning, intermediate, and final periods of intense spraying activity. DNA damage was significantly different among groups; no concurrent control was used. When the leukocytes of farmers were analyzed after a 1-day spraying period, a significant effect was observed only in subjects exposed to a fungicide (chlorothalonil)–insecticide mixture (*43*).

In greenhouse workers exposed to mixtures of pesticides, higher MN frequencies were detected in a subset of workers with work histories of extensive pesticide spraying compared with other exposed subjects and controls (*44*). In another group from Italy, DNA damage was analyzed by a ^{32}P-postlabeling assay. The DNA adducts were significantly higher in floroculturists compared with controls ($p < 0.001$). These findings support the use of the ^{32}P-postlabeling assay in agricultural studies (*45*). In sprayers involved in a Mediterranean fruit fly eradication program, Titenko-Holland et al. (*46*), using a micronucleus assay, observed no effect of malathion exposure in lymphocytes.

In phosphate fertilizer factories, workers are exposed mostly to fluorides. The frequencies of CAs and MN were higher in workers than in controls (*47*).

Welders are exposed to chromium and nickel. Exposure to these metals was measured in blood samples. With a chromium concentration in erythrocytes of 4.3 ± 7.0 mg/L and a nickel concentration in blood of 4.6 ± 1.4 mg/L, increased SCEs were observed in welders compared with controls ($p = 0.04$) (*48*).

The genotoxic effect of occupational exposure to cytostatics was investigated in three studies. Rubeš et al. (*49*), using FISH with painting probes specific for chromosomes 1 and 4, found significant differences in the number of translocations ($p < 0.01$), as well as in CAs determined by conventional methods, between an exposed group and controls ($p < 0.05$). Burgaz et al. (*50*) observed in a group of nurses an increase of MN frequencies in peripheral lymphocytes and in buccal epithelial cells. Using the Comet assay, Ündeger et al. (*51*) found that DNA damage was greater in nurses than in controls and that this effect was smaller in nurses using individual safety protection during their work.

Sardas et al. (*52*) used the Comet assay to detect DNA damage in the lymphocytes of hospital operating personnel who were exposed to anesthetic gases. The operating room personnel had substantially greater damage to their lymphocytes than did controls. The extent of damage in exposed smokers was significantly higher than that in exposed nonsmokers.

An interesting exposure was analyzed in cigarette factory workers exposed to tobacco dust (*53*). The lymphocyte DNA damage analyzed by the Comet assay showed that the exposed workers had a larger tail moment than controls ($p < 0.05$) and that smokers had significantly larger tail moments than nonsmokers. This study suggests a synergistic effect of tobacco dust exposure and smoking on DNA damage.

It is believed that airline pilots and crew members may be affected by ionizing radiation of cosmic origin. Romano et al. (*54*) observed an increase of dicentric and ring chromosomes in the peripheral blood lymphocytes of flight personnel. In another study with flight engineers, an increase of oxidative damage as determined by 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) was observed. Chromosomal aberrations, micronuclei, and HPRT mutations were not significantly increased (*55*).

In a limited number of the occupational studies mentioned above, biomarkers of susceptibility for evaluating the effect of genotypes on biomarkers of exposure and effects were also analyzed (Table 1).

In the group of Czech and Slovak coke oven workers with elevated exposure to PAHs, the effects of *GSTM1* and *NAT2* polymorphisms on DNA adducts (*6*), CAs, SCEs (*7*), and Comet assay parameters (*8*) were studied. No effect of either genotype was observed on any of these biomarkers. Pan et al. (*9*) analyzed polymorphisms of *CYP1A1 Ile/Val* in exon 7 and *GSTM1* polymorphisms. *CYP1A1* and *GSTM1* genotypes were unrelated to 1-OH-pyrene levels. DNA adducts correlated with *CYP1A1 Ile/Val* or *Val/Val* polymorphisms, but no effect of the *GSTM1* null polymorphism on the DNA adduct levels was observed. No effect of either genotype was observed on serum p53 protein levels. Wu et al. (*10*) showed the effect of *CYP1A1 Msp*I genotype polymorphism on 1-OH-pyrene concentrations in urine. Subjects with the homozygous variant genotype had a two-fold higher postshift of 1-OH-pyrene levels. This indicates that a *CYP1A1 MspI* variant genotype can modify the metabolism of PAHs in coke oven workers.

In the group of foundry workers studied by Hemminki et al. (*14*) neither *GSTM1* or *CYP1A1* genotypes affected the level of DNA adducts determined by ^{32}P-postlabeling. The authors suggested that the effect of genotypes in their study should not be used as negative evidence because the exposure to PAHs was low.

In another group of soldiers exposed to PAHs from oil well fires, the effect of *CYP1A1* (*Msp*I) and *GSTM1* and *GSTT1* polymorphisms were analyzed. No increase in urinary 1-OH-pyrene level or DNA adducts by immunoassay or by ^{32}P-postlabeling was observed in any of these genotypes (*20*).

No effect of *GSTM1* or *GSTT1* polymorphisms was observed on Comet assay parameters in female shoe workers exposed to toluene and other organic solvents (*34*).

Au et al. (*41*) analyzed the effect of *CYP2E1*, *GSTM1*, *GSTT1*, and paraoxonase (*PON*) polymorphisms on CAs induced by mixed pesticides. No effect of any of these genotypes on CAs was observed; however, *CYP2A1*, *GSTM1*, and *PON* increased chromosomal aberrations using a challenge assay. The farmers who had unfavorable metabolizing alleles were more susceptible to the genotoxic effects of pesticides. Falck et al. (*44*) observed no effect of *GSTM1*, *GSTT1*, or *NAT2* genotypes on MN frequency in pesticide-exposed greenhouse workers. In another study by Scarpato et al. (*56*) with greenhouse workers, a lack of *GSTM1* did not increase chromosomal aberrations in pesticide-exposed subjects, except in the subset of smokers. Chromosomal aberrations were also increased in individuals who carried simultaneously *GSTM1* null and *GSTT1* null genotypes.

Environmental Exposure to Mutagens and Carcinogens

Table 2 summarizes the effect of exposure and genotypes on biomarkers of exposure and effect on environmentally exposed populations. Bus drivers and postal workers have been used as model groups for air pollution in big cities. A large study was organized in Denmark analyzing several end points and exposure to PAHs (*57*). The exposure was evaluated by determination of 1-OH-pyrene in urine and exposure dose by DNA and protein adducts. Significantly higher levels of bulky aromatic DNA adducts were observed in bus drivers working in the central part of Copenhagen than in bus drivers from rural or suburban areas ($p = 0.012$). In contrast, significantly higher levels of malondialdehyde in plasma and PAH-albumin adducts were observed in a suburban group ($p = 0.016$) that was used as a control group because of lower exposure to ambient air pollutants. The

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Table 2. Effect of exposure and genotypes on biomarkers of exposure and effects by environmentally exposed populations.

Group/ sample size	Exposure		Effect of exposure on biomarkers								Effect of biomarkers of susceptibility	Ref.
	Type	Measured	Urine[a]	DNA adducts[b]	Protein adducts[c]	Comet	CAs	SCEs	MN	HPRT		
Bus drivers; postal workers (control)												
Exp = 107; C = 102	PAHs, VOCs	No	No E	E	E	–	–	–	–	–	–	(57)
Exp = 106; C = 101	PAHs, VOCs	No	–	–	–	–	No E	–	–	–	*GSTM1* E *NAT2* E	(59)
Bus drivers	VOCs											
Exp = 57		Personal	–	E	–	–	–	–	–	–	–	(60)
Children	Ozone											
Exp = 87; C = 12		*Ambient*	–	E	–	E	–	–	–	–	–	(61)
Students	Ozone											
Exp = 42		Ambient	–	–	–	E	–	–	–	–	–	(62)
General populations												
Exp = 65	Pyrene	Personal	No E	–	No E	–	–	–	–	–	–	(63)
Exp = 22; C = 40	PAHs	Ambient	No E	–	–	–	–	–	–	–	–	(64)
Women	PAHs											
Exp = 51		Personal	E	No E	–	–	–	–	–	–	*GSTM1* E *NAT2* E	(101)
Mothers	PAHs											
Placenta												
Exp = 93; C = 65		Ambient	–	E	–	–	No E	–	–	–	*GSTM1* E *NAT2* E	(65) (66)
Mothers	PM10											
Venous blood												
Exp = 54; C = 20		Ambient	–	–	–	–	No E	–	–	–	–	(66)
Cord blood												
Exp = 86; C = 29		Ambient	–	–	–	–	No E	–	–	–	–	(66)
Mothers												
Venous blood	PM_{10}											
Exp = 322; C = 220		Ambient	–	–	–	No E	–	–	–	–	*GSTM1* No E	(69)
Cord blood	PM_{10}											(69)
Exp = 322; C = 220		Ambient	–	–	–	No E	–	–	–	–	*GSTM1* No E	
Mothers												
Venous blood	PM_{10}											
Exp = 70		No	–	E	–	–	–	–	–	–	*GSTM1* No E *CYP1A1* E	(70)
Cord blood	PM_{10}											
Exp = 70		No	–	E	–	–	–	–	–	–	*GSTM1* No E *CYP1A1* No E	(70)
Mothers	PAHs											
Placenta												
Exp = 70; C = 90		No	–	No E	–	–	–	–	–	–	*CYP1A1* E	(71)
Mothers	PCBs, PCDFs											
Placenta												
Exp = 52; C = 30		No	–	E	–	–	–	–	–	–	–	(73)
Traffic police												
Exp = 34; C = 36	*B[a]P*	Personal	–	E (*summer*)	–	–	–	–	–	–	–	(74)
Exp = 54; C = 35	PAHs	Personal	–	–	–	–	–	No E	–	–	–	(75)
Exp = 82; C = 34	PAHs	Personal	–	–	–	–	–	–	No E	–	–	(76)
Exp = 94; C = 52	PAHs	Personal	No E	–	–	–	–	–	–	–	*GSTM1* No E *GSTT1* No E *CYP1A1* No E	(77)

(Continued)

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Table 2. (*Continued*)

Group/ sample size	Exposure Type	Exposure Measured	Effect of exposure on biomarkers: Urine[a]	DNA adducts[b]	Protein adducts[c]	Comet	CAs	SCEs	MN	HPRT	Effect of biomarkers of susceptibility	Ref.
Exp = 87; C = 56	PAHs	No	–	–	–	–	–	E	E	–	–	(*78*)
General population	Crude oil											
Exp = 26; C = 9		No	–	No E	–	–	–	–	–	No E	–	(*79*)
Waste site	Uranium mining											
Exp = 24; C = 24		No	–	–	–	–	E[d]	–	–	–	–	(*80*)
Physical exercise												
Exp = 6		No	–	–	–	–	–	–	E	–	–	(*82*)
Rural population												
Exp = 31; C = 27	Arsenic	Personal in urine	–	–	–	–	E	–	E	–	–	(*83*)
Exp = 32; C = 18	Arsenic	Personal in urine	–	–	–	–	E	–	E	–	–	(*84*)
Mothers	Tobacco smoke, ETS											
Placenta												
Exp = 30		No	–	No E	–	–	–	–	–	–	–	(*85*)
Smoker	Tobacco smoke											
Exp = 57; C = 45		No	–	No E	–	–	–	–	–	–	–	(*86*)
Newborns	Tobacco smoke, ETS											
Exp = 42; C = 21		No	–	–	–	–	–	–	–	No E	–	(*87*)
Newborns	ETS											
Exp = 12; C = 12		No	–	–	–	–	–	–	–	E	–	(*88*)
Children	ETS											
Exp = 109		No	E	–	E	–	–	No E	–	–	–	(*89*)
Smokers												
Exp = 23; C = 42	Tobacco smoke	No	–	–	–	–	–	–	–	E	–	(*90*)
Exp = 55; C = 4	Tobacco smoke	No	–	E	E	–	–	–	–	–	*GSTM1* No E *NAT2* No E	(*91*)
Exp = 119; C = 40	Tobacco smoke	No	E	E	–	–	–	–	–	–	*CYP1A1* No E *GSTM1* No E	(*92*)
Exp = 54; C = 5	Tobacco smoke	No	–	E	–	–	–	–	–	–	–	(*16*)
Exp = 33; C = 64	Tobacco smoke	No	–	E	–	–	–	–	–	–	–	(*93*)
Exp = 9; C = 12	Tobacco smoke	No	–	E	–	–	–	–	–	–	–	(*94*)
Exp = 20; C = 20	Tobacco smoke	No	–	E	–	–	–	–	–	–	–	(*95*)
Exp = 427; C = 823	Tobacco smoke	No	–	–	–	–	–	E	No E	–	–	(*97*)
Exp = 147	Tobacco smoke	No	–	–	–	–	–	E	–	–	–	(*13*)
Exp = 47; C = 40	Tobacco smoke	No	–	–	–	No E	–	–	–	–	–	(*98*)
Exp = 40; C = 40	Tobacco smoke	No	–	–	–	E	–	–	–	–	–	(*99*)
Smokers	Marihuana											
Exp = 17; C = 17		No	–	–	–	–	–	–	–	E	–	(*100*)

[a]Metabolites in urine. [b]By ^{32}P-postlabeling or ELISA. [c]Hemoglobin or albumin adducts. [d]Effect on CAs only using challenge assay.

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DNA adduct levels in postal workers were similar to the levels in suburban bus drivers. Autrup et al. (*57*) recommended analyzing oxidative DNA damage as a biomarker of air pollution caused predominantly by diesel exhaust particles. In a subsample of bus drivers and postal workers, personal monitors for evaluation of exposure to naphthalene and fenanthrene in inhaled air were used (*58*). In the same groups the frequency of CAs in peripheral lymphocytes was determined. There was no significant difference between bus drivers and postal workers (*59*). The oxidative DNA damage in another subset of bus drivers was determined by urinary excretion of 8-oxodG (*60*). A comparison of drivers in the city center and in rural and suburban areas of Copenhagen found a significant difference in 8-oxodG excretion ($p < 0.005$). This increased excretion of 8-oxodG suggests that exposure to ambient air pollution may cause oxidative damage to DNA (*60*).

In Mexico City, Mexico, the specific air pollution problem is related to the exposure of a complex mixture accompanied by a high level of ozone. Calderon-Garciduenas et al. (*61*) examined DNA damage in the nasal respiratory epithelium of children. In the nasal cells 8-hydro-2′-deoxyguanosine (8-OHdG) and Comet assay parameters were analyzed. They observed a significant increase of single-strand breaks as well as 8-OHdG levels in exposed children compared to those in controls ($p < 0.05$). The combination of 8-OHdG and Comet assay parameters is thought to be useful for monitoring oxidative DNA damage in populations exposed to air pollution. Using the Comet assay, Valverde et al. (*62*) analyzed DNA damage in leukocytes and buccal and nasal epithelial cells in samples from young adults living in Mexico City. Increased tail image length was observed in blood leukocytes ($p < 0.05$) and nasal epithelial cells ($p < 0.001$) in young adults from the southern part of the city who were exposed to high levels of ozone compared with young adults from the northern part who were exposed to hydrocarbons and particles. These differences were not observed in buccal epithelial cells.

The seasonal effect of exposure to PAHs adsorbed to air particles was assessed by measuring personal exposure to pyrene, urinary excretion of 1-OH-pyrene, and hemoglobin BPDE adducts in Milan, Italy (*63*). There were no significant differences in the urinary levels of 1-OH-pyrene between summer and winter samples. Hemoglobin adducts were higher in winter than in summer, but the difference between seasons was not significant.

Vyskocil et al. (*64*) used 1-OH-pyrene in urine to evaluate exposure to PAHs in two areas with different levels of air pollution during the winter and summer. They did not observe any relationship between pyrene concentration in the air and 1-OH-pyrene levels in urine.

The biomarkers in pregnancy outcome studies were also used in projects analyzing the impact of air pollution on populations in Northern Bohemia in the Czech Republic and Silesia in Poland. A study of the effect of environmental pollution on placenta DNA adducts was conducted in the Czech Republic (*65*). Air pollutant levels were evaluated from continuous ambient air monitoring. The total DNA adduct levels indicated significant differences between polluted and control regions ($p = 0.04$). Higher DNA adduct levels were found in smoking mothers than in nonsmoking mothers. Cytogenetic analysis of peripheral lymphocytes from venous and cord blood of pregnant women revealed no differences among women from different regions. Multiple regression analysis indicated that DNA adduct levels in placentas were affected by the concentration of PAHs during the last month of pregnancy, active and passive smoking, or plasma vitamin C levels. DNA adducts were also significantly increased in the placentas of newborns with intrauterine growth retardation ($p < 0.005$) (*66*). Intrauterine growth redardation is thought to be induced by PM_{10} (particulate matter < 10 μm) as early as the first month of pregnancy (*67*). The DNA adduct data in placentas are complementary with *in vitro* studies of DNA binding activity and embryotoxicity (*68*) that demonstrated the genotoxic and embryotoxic potential of organic extracts of PM_{10}.

The impact of environmental air pollution (PM_{10}) on hospitalized pregnant women in the same regions was also analyzed using the Comet assay. In this study, 322 pregnancies from a polluted district and 220 pregnancies from a control district were compared for detection of DNA damage in peripheral WBCs. The results obtained for mothers and their children did not differ between those living in polluted and control districts. No effects of prematurity, ethnicity, or smoking were observed for any of the Comet parameters (*69*).

Whyatt et al. (*70*) studied the relationship between ambient air pollution and DNA adducts in maternal and cord WBCs in mothers and newborns from Cracow, Poland. Splitting this group according to low, medium, and high air pollution levels (PM_{10}), there was a dose-related increase of DNA adduct levels with ambient air pollution and the mothers' places of residence ($p < 0.05$). When the same cohort was compared with another group with lower air pollution exposure, no effect was seen on PAH–DNA adducts in the placenta (*71*).

Perera et al. (*72*) showed in the studies in Poland that ambient air pollution was significantly associated ($p < 0.05$) with the levels of PAH–DNA adducts in white blood cells from both the maternal and infant cohorts. Newborns with elevated DNA adducts in cord blood had significantly decreased birth length, birth weight, and head circumference compared to newborns with lower DNA adduct levels detected in the cord blood.

Studies from both Poland and the Czech Republic indicate a relationship between ambient air pollution and an increase in DNA adduct levels in the DNA from maternal and cord blood and/or from placenta as well as the relationship of these biomarkers to the development of newborns.

DNA adducts were also analyzed in the placenta of women environmentally exposed to polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans (PCDFs) in Canada. DNA adducts were increased in the group of Inuit women with higher organochlorine exposure (probably due to consumption of species from the marine food chain) compared with those in a reference group from Quebec city center (*73*).

The effect of environmental pollution has been also studied in several groups of traffic policemen in Italy. Peluso et al. (*74*), using personal monitors and analysis of DNA adducts in WBCs by ^{32}P-postlabeling, conducted a study to determine B[*a*]P exposure. Higher exposures to B[*a*]P and higher DNA adduct levels in the police officers were observed during the summer; no effect was observed in the winter.

Another group of policemen was studied by Bolognesi et al. (*75*). Exposure to 3.4 ng/m^3 B[*a*]P did not increase SCEs in the policemen compared to controls. In the same group of policemen, no increase of MN was observed (*76*). Merlo et al. (*77*) investigated the relationship between exposure to ambient air PAHs and urinary excretion of 1-OH-pyrene. With personal exposure higher than 3.67 ng/m^3 B[*a*]P, urinary excretion of 1-OH-pyrene was associated with cigarette smoking more than with air PAH concentration.

The effect of exposure to automobile exhaust was also studied in China by analyzing MN and SCEs (*78*). Micronuclei as well as SCEs were significantly higher in traffic policemen than in controls ($p < 0.05$). PAH exposure was not determined.

Cole et al. (*79*) analyzed the impact of exposure to crude oil after the wreck of an oil tanker. An effect of genotoxic exposure on the local population was followed by DNA adduct analysis using ^{32}P-postlabeling and an analysis of HPRT mutations. No effect of exposure was observed in this study.

Au et al. (*80*) studied residents exposed to uranium mining waste. They found an increase of CAs using a challenge assay ($p < 0.05$), which showed a significantly abnormal DNA repair response due to exposure to radioactive contaminants.

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Hartmann et al. (*81*) already showed that exhaustive physical exercise caused significant DNA damage as measured by the Comet assay. Schiffl et al. (*82*) observed an increased level of micronuclei in six volunteers running exhausting sprints, 24 and 48 hr after exercise ($p < 0.01$). These results indicate that exhausting physical exercise causes severe mutations at the chromosomal level in blood lymphocytes.

Rural populations are sometimes greatly exposed to arsenic via drinking water. In a group in Mexico in which arsenic in drinking water in exposed individuals was 408 µg/L versus 30 µg/L in controls, significant increases of CAs in lymphocytes and MN in epithelial cells from oral mucosa and from urothelial cells were found (*83*). In another study from Finland with the almost the same exposure to arsenic in drinking water (410 µg/L), an increased frequency of CAs was also observed in the exposed group (*84*).

Several studies analyzed the effect of active and passive (environmental tobacco smoke [ETS]) smoking on various groups of populations. Daube et al. (*85*) analyzed DNA adducts in human placenta (8-OHdG) in relation to tobacco smoke exposure. There was no difference in placental levels of 8-OHdG between smokers and nonsmokers. Vitamin E appears to have a protective effect on placental 8-OHdG formation.

The determination of 8-OHdG in the DNA of leukocytes from healthy donors was used as a biomarker for oxidative DNA damage (*86*). Surprisingly, smokers showed lower mean 8-OHdG levels than nonsmokers. The inverse relationship between smoking status and 8-OHdG levels was explained by the presence of an efficient repair process for the oxidative damage induced by smoking. It was concluded that 8-OHdG levels in leukocytes may not prove to be a sensitive marker of exposure to tobacco smoking.

An HPRT cell-cloning assay was used to determine mutation frequency in fetal T-lymphocytes exposed *in utero* to maternal active and passive smoking. Exposure to active and passive maternal cigarette smoking did not result in a significant increase in somatic mutation frequency *in utero* (*87*). In a subsequent study, Finette et al. (*88*) characterized gene mutations with deletions in cord blood T-lymphocytes associated with passive maternal exposure to tobacco smoke. Analysis of 30 HPRT mutant isolates from 12 newborn infants born to mothers with no evidence of environmental exposure to cigarette smoke, and 37 HPRT mutant isolates from 12 newborn infants born to mothers exposed to passive cigarette smoke showed a significant difference in the HPRT mutational spectrum in those exposed *in utero* to cigarette smoke. In particular, there was an increase in "illegitimate" genomic deletions mediated by V(D)J recombinase, a recombination event associated with hematopoetic malignancy in early childhood.

The effect of exposure to ETS was analyzed in preschool children by evaluating cotinine in urine, 4-aminobiphenyl (4-ABP) and PAH–protein adducts, and SCEs as biomarkers. ETS-exposed children had significantly higher levels of cotinine, 4-ABP-hemoglobin adducts, and PAH–albumin adducts ($p < 0.05$) than unexposed children (*89*).

Ammenheuser et al. (*90*) used the autoradiography mutant lymphocyte assay of HPRT mutations to analyze differences between smokers, former smokers, and nonsmokers. Smokers had a 4 times higher HPRT mutant frequency than former smokers or subjects who had never smoked ($p < 0.05$). This study demonstrated the sensitivity of the autoradiography HPRT assay and indicated that this assay is more likely to detect the effects of recent rather than past exposure to tobacco smoke. Dallinga et al. (*91*) analyzed 4-ABP-hemoglobin adducts and aromatic-DNA adducts in the lymphocytes of 55 smokers and 4 nonsmokers. The levels of both adducts were related to the number of cigarettes smoked per day.

The effect of smoking was also investigated by Mooney et al. (*92*). They analyzed PAH–DNA adducts by enzyme-linked immunosorbent assay (ELISA). These adducts were inversely correlated with plasma levels of retinol, β-carotene, and α-tocoferol. α-Tocoferol had a significant protective effect on DNA adducts when β-carotene levels were low. This result suggests that several micronutrients may act in concert to protect against DNA damage and highlights the importance of assessing overall antioxidant status to evaluate DNA damage induced by various genotoxicants.

Using ^{32}P-postlabeling for analysis of DNA adducts in WBCs and alveolar macrophages of smokers, Van Schooten et al. (*16*) observed significant correlations between the number of cigarettes smoked per day and the level of aromatic DNA adducts analyzed in lymphocytes. With higher exposure levels, less efficient DNA adduct formation was observed. This led to a nonlinear dose–response relationship.

Romano et al. (*93*) evaluated PAH–DNA adducts in oral mucosa cells by an immunohistochemical assay using a specific antiserum against B[*a*]P–DNA adducts. They found that in smokers, PAH–DNA adducts were significantly increased with the number of cigarettes smoked per day ($p < 0.05$). Using nasal epithelium cells (*94*), DNA adducts measured by a ^{32}P-postlabeling assay were significantly increased in smokers compared with nonsmokers ($p < 0.001$). This finding suggests that the level of DNA adducts measured from biopsies of the nasal mucosa seems to be a reliable marker of exposure to cigarette smoking. Using a monoclonal antibody for 4-ABP– and BPDE–DNA adducts in oral mucosa and urothelial cells, smoking significantly increased both DNA adducts in both type of cells (*95*).

To evaluate SCEs and MN, Barale et al. (*96*) organized a population study in 1,650 subjects of Italy. SCE was linearly correlated with the number of cigarettes smoked per day, but no increase in MN frequency was observed (*97*). A similar effect of smoking was observed in groups from Poland in men environmentally exposed to ambient air pollutants. Smoking was a major factor influencing the level of SCEs (*13*).

Using the Comet assay in a population exposed to chronic low irradiation, Wojewódzka et al. (*98*) observed no effect of smoking habits even when using specific endonucleases as an indicator of oxidative damage. The effect of smoking was not seen in either direct DNA strand breakage and alkali-labile lesions or in enzyme oxidative determinations.

Piperakis et al. (*99*) studied the effect of smoking on hydrogen peroxide (H_2O_2)-induced oxidative damage measured by the Comet assay in peripheral lymphocytes. Smoking significantly increased the response of lymphocytes to H_2O_2, especially in males 20–25 years of age compared with nonsmokers ($p < 0.001$).

Surprisingly, of the 12 studies on the effect of tobacco smoke on biomarkers of exposure and effect, only 2 studies evaluated smoking status using cotinine levels in plasma (*16,91*).

Amenheuser et al. (*100*) also analyzed the frequencies of HPRT mutants in lymphocytes of marihuana-smoking mothers and theirs newborns. The frequency of variant lymphocytes in marihuana smokers was significantly higher than in controls ($p < 0.001$). Similarly, higher levels of HPRT mutations were observed in newborns of mothers who smoke marihuana than in newborns of nonsmokers ($p < 0.05$). The study indicates that marihuana smokers may have an elevated risk of cancer and that smoking marihuana while pregnant may affect the fetus, resulting in a high risk of birth defects or childhood cancer.

There are a limited number of studies on the effect of genotype on biomarkers of exposure and the effect on environmentally exposed groups (Table 2).

Knudsen et al. (*59*) analyzed the effect of *GSTM1* and *NAT2* genotypes on the frequency of CAs in bus drivers and postal workers. Bus drivers with the *GSTM1* null genotype and NAT2 slow acetylators had increased frequencies of cells with CAs.

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Costa et al. (*101*) examined the interaction of *GSTM1* and *NAT2* genotypes and personal exposure to carcinogenic PAHs with urinary PAH metabolites and DNA adducts in the WBCs of women working in outside environments. Urinary PAH metabolites increased for individuals with NAT2 slow acetylators and the combination of GSTM1 null and NAT2 slow acetylators.

A study of mothers from regions with different annual average air pollution levels of PM_{10} and PAHs in the Czech Republic showed that higher DNA adduct levels were detected in the group with a *GSTM1* null genotype than in the group with a *GSTM1* positive genotype ($p = 0.018$). This finding was more pronounced among the residents of the polluted district (*65*). Using multiple regression analysis in the same cohort, DNA adduct levels in placentas were also affected by *NAT2* genotype (*66*). Šrám et al. (*69*) analyzed the effect of *GSTM1* genotype on Comet assay parameters in maternal venous blood and cord blood. No effect of *GSTM1* polymorphism was observed.

In a cohort of 70 mothers and newborns from Poland, DNA adducts in maternal WBCs were not related to *CYP1A1 Msp*I or *GSTM1* polymorphisms, but DNA adducts were significantly higher in newborns who were heterozygous or homozygous for the wild-type genotype of *CYP1A1 Msp*I (*70*). An investigation of the relationship between PAH–DNA adduct levels and *CYP1A1 Msp*I polymorphisms showed that placental PAH–DNA adduct levels were also significantly higher in newborns who were homozygous or heterozygous for the wild-type of *CYP1A1 Msp*I (*71,73*).

The effect of *CYP1A1, GSTM1,* and *GSTT1* genotypes on urinary excretion of 1-OH-pyrene was analyzed in a group of traffic policemen (*77*). No significant role was detected for any metabolic polymorphisms.

4-ABP–hemoglobin adducts and aromatic DNA adducts in smokers were analyzed relative to *GSTM1* and *NAT2* polymorphisms. No influence of *GSTM1* and *NAT2* polymorphisms on hemoglobin adduct formation was observed. Higher levels of DNA adducts in lymphocytes were observed in NAT2 slow acetylators than in intermediate acetylators ($p = 0.05$) (*91*).

The effect of smoking was examined in the study of 159 heavy smokers enrolled in a smoking cessation program (*92*). Smokers with CYP1A1 exon 7 valine polymorphisms had significantly higher levels ($p < 0.03$) of DNA adducts than those with no polymorphisms. There was no effect of GSTM1 or interaction between *CYP1A1 Msp*I and *GSTM1* genotypes with respect to DNA adduct levels.

Discussion

It is difficult to evaluate molecular epidemiology studies, as some studies do not fulfill the well-known paradigm of environmentally induced cancer, beginning with a request for exposure data (*3*). From the 47 studies presented in Table 1 on the effect of exposure in occupationally exposed groups, data on personal exposure were obtained for 22 studies (47%) and data on ambient exposure in 3 studies (14%). Personal monitoring was used to determine the exposure to PAHs, VOCs, benzene, 1,3-butadiene. and epichlorohydrine.

1-OH-pyrene appears to be an effective biomarker of occupational exposure to PAHs (*9,10,20*).

DNA adducts measured by ^{32}P-postlabeling were a sensitive biomarker of exposure to PAHs in coke oven (*6*) and foundry (*14*) workers but not in another study of coke oven workers (*9*). This indicates the necessity to use a standardized procedure for ^{32}P-postlabeling, as proposed for interlaboratory trials by Phillips and Castegnaro (*102*). DNA adducts were also a sensitive biomarker of exposure to BD in the monomer production unit (*38*) and to a pesticide mixture in greenhouse floriculturists (*45*).

Protein adducts were determined as hemoglobin or albumin adducts. In all three studies that determined the effect of exposure to PAHs in coke oven workers (*11*), to nitro-PAHs in bus garage workers (*21*), or to epichlorohydrin in factory workers (*40*), no effects of exposure on these protein adducts were observed.

The Comet assay was used in 11 studies. Effects of exposure were observed with VOCs (*23*), hydrocarbons (*25*), benzene (*27*), with a mixture of pesticides in farmers (*42,43*), cytostatics (*51*), anesthetic gases (*52*), and tobacco dust (*53*). No effects were observed with exposure to PAHs in coke ovens (*8*), organic solvents (*34*), or BD (*37*). Collins et al. (*103*) pointed out " . . . its application to human biomonitoring was realized very quickly and, perhaps prematurely . . . essential questions remain concerning the reliability and reproducibility of the assay." The Comet assay became a very popular method during the last 6–8 years. To be used properly, it needs international standardization. There are discrepancies in the time of isolation of cells, in the evaluation of Comet parameters between computer-based image analysis and visual scoring, and in the statistical analysis of data. Because strand breaks are quickly rejoined by cellular processes, modification of the Comet assay by including digestion with lesion-specific endonucleases seems to be preferable as the method of evaluating cellular DNA repair (*104*). It is probably premature to use the results of the Comet assay for risk assessment, as the significance of the induced changes is not well understood.

Cytogenetic analysis of chromosomal aberrations in peripheral lymphocytes has already been used for 35 years as a biomarker of exposure to carcinogens. The significance of CAs increased with the Nordic and Italian studies (*105,106*) that showed the relationship between their levels and the risk of cancer. They were determined in 13 studies. In 11 studies effects of exposure to PAHs in coke ovens (*7*), hydrocarbons (*25*), benzene (*31,32*), acrylonitrile together with dimethyl formamide (*35*), BD (*37,39*), pesticides in farmers (*41*), fluorides (*47*), cytostatics (*49*), and cosmic radiation (*54*) were observed. Only in two studies, greenhouse workers exposed to pesticides (*44*) and crews exposed to cosmic radiation (*55*), were no effects of exposure found. Positive effects with exposure to BD (*39*) and pesticides (*41*) were observed using a challenge assay, indicating some abnormalities in the DNA repair response.

SCEs were analyzed in 13 studies. Relationships to exposure were observed with PAHs (*7,13,18,19*), acrylonitrile with dimethyl formamide (*35*), styrene (*36*), BD (*37*), epichlorohydrin (*40*), and Cr and Ni (*48*). No effects were found with exposure to VOCs (*23*), hydrocarbons (*25*), or benzene (*22,28*).

MN were determined in 16 studies. Effects of exposure were observed with PAHs (*18,19*), benzene (*28*), epichlorohydrin (*40*), pesticides (*44*), fluorides (*47*), cytostatics (*50*), and cosmic radiation (*54*). No effects were induced by VOCs (*23,24*), benzene (*26,33*), styrene (*36*), BD (*37*), malathion (*46*), or cosmic radiation (*55*).

In comparing the sensitivities of CAs, SCEs, and MN, it appears that CAs and SCEs are more sensitive biomarkers than MN.

Other biomarkers of effect were used only infrequently: HPRT was determined in 3 studies, but no effects of exposure to PAHs (*12*), epichlorohydrin (*40*) or cosmic radiation (*55*) were observed. The induction of p53 protein levels was affected by PAHs in coke oven workers (*9*), but no effect of VOCs on p21 protein levels in airport personnel was observed (*23*).

As biomarkers of susceptibility, the genotypes *CYP1A1, CYP2E1, GSTM1, GSTT1, NAT2,* and *PON* were determined. DNA adducts (*9*) and 1-OH-pyrene (*10*) by *CYP1A1* polymorphism were increased with exposure to PAHs in coke oven workers. Genetic polymorphisms of *CYP2E1, GSTM1,* and *PON* affected the frequency of CAs using a challenge assay (*41*). No effects of *GSTM1, NAT2,* and/or *CYP1A1* were observed with exposure to PAHs in coke ovens (*6–14*) and oil well fires (*20*), to organic solvents (*34*) or to pesticides (*44,56*).

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Conclusions are unexpected: the impact of occupational exposure to mutagens and carcinogens on the level of biomarkers of exposure and effect is not significantly influenced by the genetic polymorphisms that until now have been used in these studies. These results of the effect of genotypes in groups highly exposed to PAHs correspond to those of Vineis and Martone (*107*) who suggest that the effect of genotype is more pronounced at low doses and that individual susceptibility is irrelevant under exceptionally high exposure conditions.

In many cases, studies on environmental exposure to mutagens and carcinogens lack data on exposure. It is sometimes difficult to relate the observed effects only to air pollution if information on ambient exposure and life style are not fully presented (*108*). For example, Vyskocil et al. (*64*) did not observe any relationship between pyrene levels in air and 1-OH-pyrene levels in urine. Therefore, they concluded that PAHs in food probably contribute to masking the influence of air pollution on urinary 1-OH-pyrene. Yet no information about diet was included in this study, which could lead to the misinterpretation that human exposure to PAHs is predominantly from dietary sources (*109*).

The effects of exposure and genotypes on biomarkers of exposure and effect by environmentally exposed populations were analyzed in 41 studies. Data on personal exposure were collected in 9 studies (22%) and on ambient exposure in 5 studies (13%).

1-OH-pyrene levels were determined to be biomarker of exposure to PAHs, in 4 studies (*57,63,64,77*). No effects of exposure to PAHs in ambient air on 1-OH-pyrene levels in urine were observed for exposure to PAHs in ambient air. According to these results, 1-OH-pyrene is not a very sensitive biomarker of environmental exposure to PAHs. Our conclusion contradicts the recent evaluation by Dor et al. (*110*), who claimed that 1-OH-pyrene is the most relevant biomarker for estimating individual exposure to environmental pollution.

The only positive results were those by Costa et al. (*101*); they determined a total of 28 PAH/metabolites in urine including the following parent PAHs and their hydroxylated metabolites: anthracene, B[*a*]P, chrysene, pyrene, methylchrysene, and methyl B[*a*]P.

DNA adducts were determined in 18 studies. The ^{32}P-postlabeling method was used in 7 studies. DNA adducts were related to PAH exposure in bus drivers (*57*), in placentas (*65,66*), in traffic policemen (*74*), and in smokers (*16,91*). Two studies found no effect of PAH exposure on DNA adduct levels (*79,101*). Immunologic methods such as competitive ELISA or immunohistochemistry use polyclonal and monoclonal antibodies to recognize PAH–DNA adducts (*111*). These methods were used in 8 studies. DNA adducts were increased in maternal venous and cord blood (*70*) and in smokers (*91–93,95*). No effect was seen for PAH exposure in placenta (*71*) or for tobacco smoke exposure in placentas (*85*) and smokers (*86*).

It is difficult to compare the ^{32}P-postlabeling and immunologic methods. Until now, immunologic methods were not internationally validated in the same way as the ^{32}P-postlabeling method (*102*). According to Santella (*111*)

> . . . the data generated by immunological methods may not be as absolutely quantitative as that obtained in other types of assay, the DNA adduct levels found in different populations provide important information on exposure monitoring . . . .

Oxidative damage was measured in 5 studies. 8-oxodG in urine was determined in bus drivers (*57,60*). One study found a negative association (*57*), another a positive one (*60*). Both results are from the same group, but it is difficult to compare the groups because Autrup et al. (*57*) expressed their results in nmol/mmol creatinine and Loft et al. (*60*) in pmol/kg 24 hr. 8-OHdG (using an immunohistochemical method) increased in children exposed to ozone (*61*); no effect of tobacco smoke was observed in placentas, using ^{32}P-postlabeling to determine 8-OHdG (*85*) or in leukocytes of smokers using high performance liquid chromatography detection (*86*).

Methods analyzing 8-oxodG and 8-OHdG were criticized because of various factors that artifactually induce oxidative DNA lesions during DNA extraction (*112,113*). Using an improved chaotropic NaI method, Helbock et al. (*114*) found that the steady-state level of 8-oxodG may be significantly reduced. Nakamura et al. (*115*), isolating DNA from cells with 2,2,6,6-tetramethylpiperidinoxyl minimized the artifactual induction of oxidative lesions during DNA extraction. Oxidative damage is understood to be an important injury of DNA. It is important to organize interlaboratory trials to determine methods that could be standardized for human monitoring.

Protein adducts were increased as PAH-albumin adducts in bus drivers (*57*) and children exposed to ETS (*89*), as 4-ABP-hemoglobin adducts in children exposed to ETS (*89*) and in smokers (*91*). No effect of PAH exposure was detected when BPDE–hemoglobin adducts were measured (*63*).

The Comet assay was used in 5 studies. An effect of exposure was observed with increased ozone exposure (*61,62*) and using *in vitro* H_2O_2-induced oxidative damage in young smokers (*99*). No effects of PM_{10} (*69*) or tobacco smoke (*98*) were observed in two studies.

Chromosomal aberrations were determined in 5 studies. The frequency of CAs was increased in studies analyzing the effect of high arsenic levels in drinking water (*83,84*) and of uranium mining waste in residents using a challenge assay (*80*). No increase was related to PAH exposure in bus drivers (*59*) or mothers and newborns (*66*).

SCE were determined in 5 studies. Effects were observed for automobile exhaust (*78*) and tobacco smoke (*13,97*); no effects of PAH exposure were seen in policemen (*75*) or in children exposed to ETS (*89*).

MN were determined in 4 studies. Their frequency was increased in policemen (*78*), by exposure to arsenic in drinking water (*83*) and by physical exercise (*82*); no effects were seen in another group of policemen (*75*).

HPRT mutations were followed in 5 studies. The mutation spectrum was changed in newborns exposed to ETS (*88*); the mutation frequency was increased in tobacco smokers (*90*) as well as in marihuana smokers (*100*) and their newborns. No effect was seen in a population exposed to spilled crude oil (*79*) or in newborns exposed to ETS (*87*).

Comparing all cytogenetic end points, there were no significant differences in their sensitivities to environmental exposure.

Biomarkers of susceptibility were determined in 10 of a total 41 studies as genotypes of *CYP1A1, GSTM1, GSTT1,* and *NAT2.*

CYP1A1 affected DNA adducts in newborns (*70*) and placentas (*71*) of residents of an air-polluted region; no effect was observed on 1-OH-pyrene in the urine of policemen exposed to air pollution (*77*) or smokers (*92*) or on DNA adducts in smokers (*92*).

A *GSTM1* null genotype increased the frequency of CAs in bus drivers exposed to PAHs (*59*), DNA adducts in the placentas of mothers exposed to PAHs (*65*), and the concentration of PAH metabolites in urine from ambient air exposure to PAHs (*101*). No effect was seen in mothers or newborns on Comet parameters (*69*) and DNA adducts (*70*), in policemen on 1-OH-pyrene in urine (as well as GSTT1) (*77*), or in smokers on DNA (*91,92*) and protein (*91*) adducts.

The *NAT2* slow acetylator genotype also affected the frequency of CAs in bus drivers (*59*), DNA adducts in placentas (*66*), and the concentration of PAH metabolites in urine (*101*). No effect was observed on the level of DNA and protein adducts in smokers (*91*).

In summary, the effects of different genotypes appear to vary in different populations because of the level of exposure to pollutants as well as the spectrum of biomarkers used.

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Conclusion

Evaluating the achievements of molecular epidemiology during the period 1997–1999 probably reveals only the tip of the iceberg, as the published papers presented the results of studies mostly from the early nineties. This also means that some studies, especially those analyzing the effects of genetic polymorphisms, were sometimes based on small sample sizes.

Several requirements for human studies in the future must be stressed. Using various biomarkers, the studies should be related to ambient and personal monitoring, and should also provide information about lifestyles. When the effect of smoking is analyzed, determining cotinine levels should be one of the basic requirements. Many studies were deficient in experimental design, using only exceptionally coded samples (blinded) and repeating the analysis of a subset of the samples that had been recorded.

DNA adducts measured by a ^{32}P-postlabeling method have become the most popular of biomarkers of exposure (*102*). They are probably the biomarker of choice for evaluating PAH exposure (*116–119*).

Protein adducts are useful as a biomarker for exposure to tobacco smoke (4-ABP) or exposure to smaller carcinogenic molecules such as, e.g., acrylonitrile, BD, or epichlorohydrine (*17*).

Use of the Comet assay still needs reliable validation for human biomonitoring (*103,104*), as at present, it appears that its use for monitoring human exposure to environmental pollurants is not fully validated.

Methods analyzing oxidative DNA lesions by GC–MS or HPLC also need reliable standardization and international validation.

Of the biomarkers of effect, the most common are cytogenetic end points. An analysis of Nordic and Italian cohorts suggests that the significance of chromosomal aberrations in peripheral lymphocytes has increased, indicating a predictive value of chromosomal aberration frequency for cancer risk (*105*). Findings from these epidemiologic studies support the use of chromosomal breakage as a relevant biomarker of cancer risk (*106*).

The impact of genetic polymorphisms as biomarkers of susceptibility is of key significance in understanding the processes of genetic damage involved in mutagenesis and carcinogenesis. Until now, there have not been sufficient data to interpret the relationship between genotypes, biomarkers of exposure, and biomarkers of effect related to risk assessment of human exposure to mutagens and carcinogens. It is possible that this process will progress rapidly with the Environmental Genome Project (*120*).

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